16. (Amended) The method of claim 15 wherein the tissues are selected from the group consisting of rat, mouse, human and non-human primate.

REMARKS

Status of the Application and the Present Amendment

Claims 1 to 21 are pending and stand rejected in the application. With entry of the present amendment, claims 5, 6, 10, and 16 have been amended. Applicants note that the claim amendments are made for improved clarity or expedition of prosecution, and should not be viewed as acquiescence of any ground of rejection unless otherwise noted. No new matter has been added by the present amendment.

The following remarks address issues raised in the Office Action.

Rejection Under 35 U.S.C. 112, 2nd Paragraph

The Office Action makes various rejections of the pending claims based on alleged indefiniteness. Each of the rejections is addressed below. As an initial matter, Applicants note that an indefiniteness rejection should not be based on reading the claim language in abstract. Rather, as stated in the MPEP:

> Definiteness of claim language must be analyzed, not in a vacuum, but in light of:
> (A) The content of the particular application disclosure;
> (B) The teachings of the prior art; and
> (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. [MPEP § 2173.02 at 2100-194]

1. Claim 1, 5, and 6 are rejected as allegedly lacking a step which relates back to the preamble. With respect to claim 1, the Office Action says that the preamble recites a method for identifying redundant clones but the last step of the claim is identifying clones for

which hybridization signal produced is different. Applicants respectfully traverse this rejection. The last step of the claim recites that "identifying clones for which the hybridization signal produced is different in the presence and absence of the isolated polynucleotide is redundant clones" (emphasis added). Thus, the claim clearly relates back to the preamble. Accordingly, the rejection should be withdrawn.

Claim 5 was similarly rejected as allegedly lacking a step that relates back to the preamble. Again, Applicants traverse. The preamble recites a method of identifying previously characterized clones in a cDNA library. The last step of the claim is "identifying clones for which the hybridization signal produced is different in the presence and absence of the isolated polynucleotide as previously characterized clones" (emphasis added). The claim thus clearly relates back to the preamble.

Claim 6 was also rejected as allegedly lacking a step that relates back to the preamble. To expedite prosecution, claim 6 has been amended which now recites "combining the first and second portions, thereby producing normalized or subtracted cDNA library." As such, the last step of the amended claim clearly relates back to the preamble. Therefore, the rejection should be withdrawn.

2. Claims 15-21 are rejected for the alleged indefiniteness in the recitation of "related tissues or cells," "driver-tissue," and "tester-tissue."

Regarding the recitation of "related tissues or cells," Applicants note that the word "related" is not a technical term that needs to be defined in order for one to understand. Rather, the word has its ordinary meaning, i.e., being connected in some aspects. Its metes and bounds become even more clear in view of the present disclosure. For example, the specification discloses that the two related tissues or cells are respectively termed "driver" and "tester" (e.g., page 1, lines 26-27). The specification also discloses that the "driver" and "tester" tissues are "pairs of tissues that are of the same type, but which differ in one major characteristic, such as disease state (e.g., diseased & normal brain tissue), age (e.g., adult and fetal liver tissue), exposure to drugs, or other state (e.g., stimulated & unstimulated; activated & unactivated), etc." and that "the tissue source may be human or non-human" (see, page 6,

lines 9-13). Thus, one of ordinary skill in the art would appreciate that the tissues are related to the extent that they are identical except for one major characteristic that is the focal point of a given analysis.

Similarly, to determine the metes and bounds of the phrases "tester-tissue" and "driver-tissue," they must be viewed by an ordinarily skilled person in the art in view of the present disclosure. First, as noted above, the specification makes clear that the pair of related tissues are designated the "tester" and the "driver." Thus, the meanings of "tester" and "driver" become clear when one takes into consideration the major characteristic that separates these two related tissues. For example, when the major characteristic is exposure to a drug, the tester and the driver are the stimulated tissue and the unstimulated tissue, respectively (see, e.g., page 13, lines 5-6). Further, the specification also discloses that it is entirely arbitrary which one of the pair of related tissues is "driver" and which is "tester" (e.g., page 7, lines 7-8). It is further noted that "driver" and "tester" tissue (or similar terms such as "target" or "tracer") are terms well known and recognized in the art. For example, in the references cited in the instant Office Action (e.g., Soares et al., U.S. Patent No. 5,846,721; and Sutcliffe et al, U.S. Patent No. 6,074,872), the term "driver," "target," or "tracer" are used in the same or similar sense as the use of "driver" and "tester" in the present invention.

In summary, the noted phrases are not indefinite because an ordinarily skilled person in the art (as opposed to a lay person), in view of the disclosures in the subject specification and the teachings of the prior art (as opposed to reading the claims in abstract), would undoubtedly understand their metes and bounds in the context of the present invention. On the other hand, it would be unreasonable to require Applicants to provide detailed definitions of these terms in the claims. Accordingly, the instant rejection should be withdrawn.

3. Claims 1-14 are rejected as allegedly indefinite because claim 1 recites a broad range or limitation and a narrow range or limitation that falls within the broad range or limitation. Specifically, the Office Action takes the position that the preamble recites "identifying redundant clones" while the body of the claim (i.e., step (a)) recites "identifying at

least one redundant clone." According to the Office Action, such recitations render the claim indefinite. Applicants traverse the rejection for the clarifications stated below.

First of all, the Office Action apparently overlooked the fact that step (e) of claim 1 clearly recites that multiple clones are identified "as redundant clones," which relates back to the preamble. In addition, step (a) is just one step of the claim. It recites identifying "at least one redundant clone" as a necessary step in order to identify more "redundant clones in later steps. The "at least one redundant clone" identified in step (a) does not correspond to the "redundant clones" to be identified as recited in the preamble. Rather, it is just one step towards achieving that goal of identifying the redundant clones. Thus, contrary to the assertion in the Office Action, the recital of "redundant clones" in the preamble and the recital of "at least one redundant clone" in step (a) do not constitute having broader range/limitation and narrow range/limitation in the same claim which would otherwise render the claim indefinite. Therefore, the instant rejection should be withdrawn.

4. Claims 1-14 are also rejected as allegedly indefinite for the recitation of "first portion" and "second portion." It was stated in the Office Action that it is not clear how portion is defined and how many portions constitute the cDNA library.

To traverse the rejection, Applicants again emphasize that in order to determine whether the claims are definite, the claims must be viewed in the context of the present disclosure and prior art teachings by one of ordinary skill in the art. As an initial matter, Applicants note that a DNA library is generally understood in the art to mean a collection of different polynucleotides (i.e., with different sequences). The library will normally contain multiple copies of each of the different polynucleotides. Such is consistent with the teachings of the subject application (see, e.g., page 5, line 15). One of ordinary skill and knowledge in the art would readily understand that, in the context of claim 1, the "first portion" will comprise part of the collection of different polynucleotide molecules, but not necessarily with each of the different polynucleotides being represented in that part. Because it is evident that the first portion of the cDNA library would comprise multiple cDNA molecules from the library, it certainly does not mean just a few nucleotides as questioned in the Office Action.

In addition, one would understand that the amount of polynucleotide molecules (i.e., cDNA molecules) in the first portion should be sufficient for one to identify at least one redundant clone. The ordinarily skilled person in the art of molecular biology only needs routine experimentation to determine the exact size of the first portion of the cDNA library in order to accomplish this goal. Thus, for example, if the entire cDNA library consists of 5 μg total cDNA with 1,000 different polynucleotides, the first portion could be of 0.1, 0.2, 0.5, or 1 μg of the total cDNA and represent 100, 200, 500, or all the 1000 different sequences, as long as it allows identification of at least one redundant clone.

Finally, it is not clear to Applicants why the claim should specify how many portions constitute the cDNA library. The claim clearly recites that one portion of the library should be used to identify at least one redundant clone before performing the other steps recited in the claim. All the other steps are also clearly set forth in the claim. Applicants simply cannot see any indefiniteness in such recitation of the claim. Should the Office persists in maintaining the rejection, clarification of the alleged indefiniteness is respectfully requested so that Applicants can better address any issue that may be present.

5. Claim 5 is rejected as missing step (c). Applicants thank the Examiner for her careful reading of the claim. The inadvertent typographical error in the claim has been corrected by renumbering the steps recited in the claim.

6. Claims 10 and 16 are rejected as allegedly using improper Markush language. Although Applicants respectfully disagree with the assertion, to expedite prosecution, the claims have been amended as suggested by the Examiner. The rejection is therefore overcome.

Rejection Under 35 U.S.C. 102(e)

At paragraph 5, the Office Action rejects claims 1-5 as allegedly anticipated by Somerville et al. (U.S. Patent No. 6,028,248). Citing to discussions in Somerville et al. at Col. 17, line 25 to Col. 18, line 67, the Office Action is of the view that Somerville et al. teach each element of claim 1. Applicants respectfully traverse this rejection.

Somerville et al. does not teach methods of claim 1-5 of the present invention. The present invention, as reflected in claim 1, teaches a method of identifying redundant clones in a cDNA library. In step (c), hybridization of a labeled probe with an array of cDNA clones takes place in the presence or absence of an isolated polynucleotide corresponding to the at least one known redundant clone. Due to competition of the added polynucleotide corresponding to the at least one known redundant clone, hybridization signals obtained from the redundant clones would be reduced as compared to hybridization signals obtained from the clones in the absence of the added polynucleotide.

By contrast, the discussion of Somerville et al. cited by the Office Action relates to differential screening of castor cDNA library for clones that are specifically expressed in seed but not in leaves. Somerville et al. used seed-specific and leave-specific probes to screen the arrayed cDNA library separately so that seed-specific clones can be identified. In addition, Somerville et al. used a probe prepared from redundant clones to facilitate screening of "seed-specific and not highly abundant" clones (i.e., by elimination of clones that hybridized to the probe corresponding to abundant clones). However, Somerville et al. do not teach hybridization of a labeled probe with a cDNA library in the presence of an isolated polynucleotide corresponding to a known redundant clone. Instead, in Somerville et al., polynucleotides corresponding to the sequenced redundant clones were used as the probe to screen the arrayed cDNA library. Unlike the present invention, they were not used in addition to a labeled probe to compete with the corresponding clones on the cDNA array for binding to the labeled probe (see, e.g., Col. 18, lines 63-65). In another word, Somerville et al. do not teach screening of an arrayed cDNA library with a labeled probe in the presence of a polynucleotide corresponding to a known redundant clone. For at least this reason, claims 1-5 are novel over Somerville et al. The instant rejection should therefore be withdrawn.

Rejection of Under 35 U.S.C. 103

1. At paragraph 7, the Office Action rejects claims 6-14 as allegedly obvious over Soares et al. (U.S. Patent No. 5,846,721) in view of Makarov et al. (U.S. Patent No. 6,197,557)

. In maintaining the rejection, the Office Action states that Soares et al. teach restricting a first portion of a double-stranded cDNA library with a first restriction enzyme and restricting a second portion of the double-stranded library with a second nuclease or restriction enzyme. The Office Action acknowledges that Soares et al. do not teach fragment sizes of within about 100-500 base pairs of each other or use of two restriction enzymes Dpn I and Rsa I, but states that such would be obvious because Soares et al. teach that the average fragment size is determined by computer implemented inspection of gene sequences from GenBank and that Makarov et al. teach use of Dpn I and Rsa I. Applicants respectively traverse the rejection. Claims 6-14 relate to methods of making a normalized or subtracted cDNA library. The methods recite use of two different restriction enzyme to respectively digest a first portion and a second portion of the same cDNA library. As for the cited art, contrary to the assertion of the Office Action, Soares et al. do not teach separate digestions of two portions from the same cDNA library with two different restriction enzymes. Rather, Soares et al. discuss methods of normalization or subtraction in which a portion of a double-stranded DNA library is digested with, e.g., a restriction enzyme to generate fragmented DNA templates for synthesis of RNAs, but a second portion is converted to single-stranded circles rather than being digested into fragments. There is simply no restriction or other enzymatic digestion of a second portion of the same DNA library. Such basic scheme of Soares et al. is clearly demonstrated for the normalization method as shown in, e.g., Fig. 2 and discussions at Col. 3, line 49 to Col. 4, line 43 in Soares et al. For the substraction methods of Soares et al., there is simply no restriction fragments being generated. Instead, in Soares et al., a substraction library (referring to as "A-B" for ease of reference) is produced by converting both the double-stranded A library and B libraries to single-stranded molecules, amplifying the single-stranded "B" library molecules, hybridizing single-stranded "B" library molecules in excess amount to single-stranded A library molecules, and separating the unhybridized single-stranded A library molecules from the hybridized molecules. The substraction library "A-B" is then generated from the unhybridized A library molecules, which enriches members of the A library that are not present in the B library. Such substraction scheme of Soares et al. is illustrated in, e.g., Fig. 4; Col. 2, line 57 to Col. 3, line 4; and Col. 4, line 44 to Col. 5, line 21 in Soares et al.

In addition, it is noted that the sections of Soares et al. that are cited in the Office Action as support for alleged teaching of restriction of a first portion of a double-stranded cDNA library (e.g., Fig. 2, Col. 3, lines 49-54) relate to the normalization methods. On the other hand, sections cited as support for alleged teaching of restriction of a second portion of the cDNA library (e.g., Col. 2, lines 60-63) actually relate to the subtraction methods. Soares et al. at most may have suggested using more than one enzyme at the same time (e.g., an endonuclease and an exonuclease) to generate single-stranded molecules (e.g., Col. 2, lines 60-63; and Col. 10, lines 38-42). However, unlike the present invention, nowhere in Soares et al. teaches or suggests use of two portions of the same DNA library with two different restriction enzymes to generate DNA fragments.

Further, in the libraries of the subject invention, the expressed genes in a tissue are represented in the libraries by a subfragment, instead of the full-size cDNA, by digesting dscDNA with a restriction enzyme to prepare the tester and driver. By contrast, the libraries discussed in Soares et al. represent full-length cDNA. In Soares et al., to normalize the library, testers which were single-stranded circular DNA molecules were prepared from plasmids of the libraries either in vivo or in vitro. The driver was prepared by (1) restriction digestion of plasmids to make linear templates which were then used in *in vitro* transcription to generate RNA driver, or (2) restriction digestion of plasmids followed by exoIII treatment to generate small DNA fragment as driver. Drivers prepared either way was then used to perform subtractive hybridization with the single-stranded circular DNA tester to normalize the library. The only purpose of Soares using restriction enzymes was to prepare the driver and not to increase the complexity of the libraries. The complexity of the library was determined when it was made.

In summary, Soares et al. discuss different methods of producing different libraries for different purposes from that of the subject invention. Specifically, unlike the presently claimed methods, the Soares et al. methods do not employ separate digestion of a first and a second portion of the same double stranded library DNA.

As for Makarov et al., this reference discusses use of a cocktail of restriction enzymes (including Rsa I and Dpn I) to produce random nicks or breaks in a double stranded

nucleic acid (Office Action, page 10, last paragraph). Makarov et al. by no means remedy the lack of teachings in Soares et al. of the above-clarified distinctive features of the subject invention.

From the forgoing, it is readily apparent that nothing in the cited art would have suggested the presently claimed methods for making a normalized or subtracted library. Withdrawal of the instant rejection is respectfully requested.

2. At paragraph 8, the Office Action rejects claims 15-21 as allegedly obvious over Sutcliffe et al. (U.S. Patent No. 6,074,872) in view of Soares et al. The Office Action says that Sutcliffe et al. inherently teach a method of comparing the quality of two different subtracted cDNA libraries each prepared from the same tester and driver RNAs, and that Sutcliffe et al. teach comparing level of hybridization of probes to the libraries, thus identifying library of higher quality. The Office Action acknowledges that Sutcliffe et al. do not teach normalized libraries, but asserts that Soares et al. teach normalized libraries. The Office Action concludes that it would have been obvious to combine teachings of Sutcliffe et al. and Soares et al., rendering the presently rejected claims obvious. This rejection is respectfully traversed for the reasons stated below.

Claims 15-20 are directed to methods for identification of clones with a desired abundance using a driver normalized library and a tester-normalized library (as reflected in step (d) of claim 15; see also discussion in the specification, e.g., at page 18), as well as clones with a desired differential expression pattern using <u>both</u> a driver-subtracted library (for down-regulated clones) and a tester-subtracted library (for up-regulated clones) (as reflected in step (e) of claim 15; see also discussion in the specification, e.g., at page 19). By contrast, Sutcliffe et al. discuss identification of a novel cortistatin sequence which is upregulated in the brain upon stimulation with high frequency. The cited discussions in Sutcliffe et al. (i.e., Columns 37-38) relate to use of a tester-subtracted library (i.e., target library subtracted with driver library). This library, which enriched clones up-regulated in the target, was used for identification of clones not present in the driver library (i.e., up-regulated in the target). Sutcliffe et al. do not discuss identification of genes that are down-regulated in the target.

There is no discussion, either expressly or inherently as alleged in the Office Action, generation of a driver-subtracted library (which enrich clones down-regulated in the target).

Soares et al. at most discussed generation of normalized libraries. They do not remedy the lack of teaching or suggestion in Sutcliffe et al. of generation of driver-subtracted libraries to enrich down-regulated clones. In addition, unlike the present claims, neither Sutcliffe et al. nor Soares et al. teach or suggest selection of clones based on a desired ratio of hybridization signals from the subtracted library with cDNA probes corresponding to mRNA from the driver tissue and the tester tissue. Thus, claims 15-21 would not have been obvious over Sutcliffe et al. in view of Soares et al.

Claim 21 is directed to a method of comparing quality of two subtracted cDNA libraries prepared from the same driver and tester tissues. The method entails comparison of level of hybridization signals from probes corresponding to the two libraries with at least one known differentially expressed polynucleotide sequence. This claim is also non-obvious over the cited art because nowhere in the cited references (e.g., Sutcliffe et al.) teach or suggest hybridization of two subtracted libraries prepared from the same driver and tester tissues with a known differentially expressed sequence and comparison of the hybridization signals. As discussed above, the cited discussion of Sutcliffe et al. (e.g., Col. 37, lines 37-49) relates only to identification of up-regulated sequences using a tester subtracted library. It does not teach or suggest comparison of signals obtained from hybridization of at least one known differentially expressed sequence with two subtracted libraries.

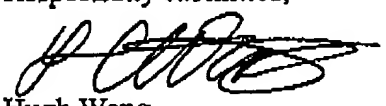
In light of the above explanations and clarifications, Applicants submit that claims 15-21 are non-obvious over the cited references and respectfully request withdrawal of the instant rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400 x 5209.

Respectfully submitted,



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Appendix Marked-up version of all claims under consideration

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PA 3178905 v2

Marked-up Version of All Pending Claims
(unamended claims appear in small font)

1. A method of identifying redundant clones in a cDNA library comprising:

(a) identifying at least one redundant clone in a first portion of the cDNA library;

(b) obtaining an isolated polynucleotide corresponding to said redundant clone;

(c) hybridizing a detectably labeled probe to an array of clones from the cDNA library, wherein said hybridizing is done in the presence and absence of the isolated polynucleotide obtained in (b);

(d) comparing the hybridization signal obtained for each arrayed clone in the presence and absence of the isolated polynucleotide; and,

(e) identifying clones for which the hybridization signal produced is different in the presence and absence of the isolated polynucleotide as redundant clones.

2. The method of claim 1 wherein the redundant clone is identified by comparing the sequences of at least 100 clones in said first portion of the cDNA library.

3. The method of claim 1 wherein the isolated polynucleotide in (d) is unlabeled.

4. The method of claim 1 wherein the isolated polynucleotide in (d) is detectably labeled.

5. (Amended) A method of identifying previously characterized clones in a cDNA library comprising

(a) obtaining an isolated polynucleotide corresponding to previously identified clones;

(b) hybridizing a detectably labeled probe to an array of clones from the cDNA library in the presence and absence of the isolated polynucleotide obtained in (a);

(c) [(d)] comparing the hybridization signal obtained for each arrayed clone in the presence and absence of the isolated polynucleotide; and,

(d) [(e)] identifying clones for which the hybridization signal produced is different in the presence and absence of the isolated polynucleotide as previously characterized clones.

6. (Amended) An improved method of making a normalized or subtracted cDNA library comprising:

(a) obtaining double-stranded cDNA (dscDNA) corresponding to mRNA from a tissue or cell;

(b) restricting a first portion of said dscDNA with a first restriction enzyme;

(c) restricting a second portion of said dscDNA with a second restriction enzyme, wherein

(i) restriction of dscDNA from the tissue or cell with the first enzyme is predicted to produce restriction fragments having a predicted average fragment size of between about 100 and about 500 basepairs;

(ii) restriction of dscDNA from the tissue or cell with the second enzyme is predicted to produce restriction fragments having a predicted average fragment size of between about 100 and about 500 basepairs; and,

(iii) the predicted average fragment size in (i) and (ii) are within about 150 basepairs of each other; and,

(d) combining said first and second portions, **thereby producing a normalized or subtracted cDNA library**.

7. The method of claim 6 wherein the predicted average fragment sizes in (i) and (ii) is between 300 and 500 basepairs.

8. The method of claim 7 wherein the predicted average fragment sizes in (i) and (ii) are within about 100 basepairs of each other.

9. The method of claim 6 wherein the tissue or cell is from a mammal.

10. (Amended) The method of claim 9 wherein the animal is **selected from the group consisting of** rat, mouse, human **and** [or] non-human primate.

11. The method of claim 10 wherein at least one of the first and second enzymes is selected from the group consisting of Alu I, Cvi RI, Dpn I, Hae III, Rsa I, Cvi J1 and Tha 1.

12. The method of claim 10 where the first enzyme is Dpn 1 and the second enzyme is Rsa 1.

13. The method of claim 6 wherein the predicted average fragment size is determined by inspection of gene sequences from Genbank.

14. The method of claim 13 wherein the inspection is computer implemented.

15. A method for selecting clones for analysis comprising:

(a) preparing double-stranded cDNA (dscDNA) corresponding to mRNA from each of a pair of related tissues or cells, wherein one member of the pair is designated the driver-tissue and the other member of the pair is designated the tester-tissue;

(b) using said dscDNA to prepare a driver-normalized cDNA library, a tester-normalized cDNA library, a driver-subtracted cDNA library, and a tester-subtracted cDNA library;

(c) hybridizing clones from each of the libraries in (b) with detectably labeled cDNA probe corresponding to mRNA from one or both of the related tissues or cells;

(d) selecting clones with a desired signal intensity from the driver-normalized cDNA library hybridized with cDNA probe from the driver tissue and the tester-normalized cDNA library hybridized with cDNA probe from the tester tissue; and,

(e) selecting clones with a desired ratio of hybridization signal from the driver-subtracted cDNA library hybridized with cDNA probe corresponding to mRNA from both of the related tissues and the tester-subtracted cDNA library hybridized with cDNA probe corresponding to mRNA from both of the related tissues.

16. (Amended) The method of claim 15 wherein the tissues are **selected from the group consisting of** **[from]** rat, mouse, human **and** **[or]** non-human primate.

17. The method of claim 15 wherein the mRNA is from a pair of tissues related as diseased tissue and healthy tissue.

18. The method of claim 17 wherein the diseased tissue is from brain.

19. The method of claim 15 wherein the diseased tissue is from an animal model of a human disease.

20. A method for selecting clones for analysis comprising:

(a) preparing double-stranded cDNA (dscDNA) corresponding to mRNA from each of a pair of related tissues or cells, wherein one member of the pair is designated the driver-tissue and the other member of the pair is designated the tester-tissue;

(b) using said dscDNA to prepare a driver-normalized cDNA library, a tester-normalized cDNA library, a driver-subtracted cDNA library, and a tester-subtracted cDNA library;

(c) hybridizing clones from each of the libraries in (b) with detectably labeled cDNA probe corresponding to mRNA from one or both of the related tissues or cells;

(d) selecting low signal clones from the driver-normalized cDNA library hybridized with cDNA probe from the driver tissue;

(e) selecting low signal clones from the tester-normalized cDNA library hybridized with cDNA probe from the tester tissue;

(f) selecting high-ratio clones from the driver-subtracted cDNA library hybridized with cDNA probe corresponding to mRNA from both of the related tissues; and,

(g) selecting high-ratio clones from the tester-subtracted cDNA library hybridized with cDNA probe corresponding to mRNA from both of the related tissues.

21. A method for comparing the quality of a two different subtracted cDNA libraries, comprising:

(a) obtaining a first subtracted cDNA library and a second subtracted cDNA library, wherein each library is prepared from the same tester and driver RNAs;

(b) preparing detectably labeled probe from DNA from each library;

(c) hybridizing said probe from each library to an array of immobilized polynucleotides, wherein at least a plurality of said polynucleotides have the sequence of genes that are differentially expressed in the tester RNA compared to the driver RNA, and detecting the hybridization of the probe to the immobilized polynucleotides;

(d) identifying at least one immobilized polynucleotide having a sequence that is differentially expressed in the tester RNA compared to the driver RNA and comparing the level of hybridization of probe from the first subtracted cDNA library to said polynucleotide with the level of hybridization of probe from the second subtracted cDNA library to said polynucleotide,

wherein, the library having the higher level of hybridization of probe to said polynucleotide is identified as a higher quality library.